EXPERT REPORT OF FRED RUSSELL KRAMER PURSUANT TO FED.R.CIV.P. 26

I have been retained as an expert witness in this action by Gen-Probe Incorporated and have agreed to testify at the trial of this action on the issue of whether the disclosures of U.S. Patent No. 5,750,338 ("the '338 patent") would have enabled one of ordinary skill in the art to achieve exponential target amplification using the enzyme Qß replicase as of the filing date of the application for the patent.

SUMMARY OF MY OPINION

I have considered the disclosures of the '338 patent, particularly with regard to the teachings of Example 7. Having reviewed the specification of the '338 patent, I conclude that the patent's disclosures completely failed to enable one of ordinary skill in the art to achieve exponential amplification using Qß replicase.

EDUCATION AND EXPERIENCE

My relevant training and experience are briefly summarized here. I received a B.S. (with honors) in Zoology from the University of Michigan in 1964. I received a Ph.D. from The Rockefeller University in 1969 and did my postdoctoral training at Columbia University from 1969 to 1972 under Dr. Sol Spiegelman. I was employed in various scientific positions from 1969 to 1986 in the Department of Genetics and Development and the Institute of Cancer Research, College of Physicians and Surgeons at Columbia University, including as a Fellow of the American Cancer Society from 1969 to 1971, a Research Associate from 1971 to 1972, an Instructor from 1972 to 1973, an Assistant Professor from 1973 to 1980, a Senior Research Associate from 1980 to 1983, and a Research Scientist from 1983 to 1986. I am a Member and Chairman of the Department of Molecular Genetics at the Public Health Research Institute and have been there since 1986. From 1987 to the present I have been a Research Professor of Microbiology and Cell Biology at the New York University School of Medicine. A true and correct copy of my resume is attached to this declaration as Exhibit "A".

I am familiar with the use of the enzyme Qß replicase in amplification methods because of my own extensive research in this area. Beginning in 1969, while doing my postdoctoral training, I worked with Dr. Spiegelman on sequencing the nucleotides of replicating RNA molecules and the study of Qß replicase. By 1983, my work demonstrated that heterologous oligonucleotides could be inserted at an appropriate site within a naturally occurring Qß template RNA, and the resulting "recombinant RNAs" could be amplified exponentially by incubation with Qß replicase. By 1992, my laboratory demonstrated that recombinant mRNAs could be amplified exponentially in this manner.

I am a co-inventor on several United States Patents in this field. The list of those patents is found in Exhibit "A".

I have stayed abreast of the general technology of amplification by regularly reviewing scientific literature and attending scientific conferences. My conclusions as provided below are based on my experience and understanding of the reactions involved in Qß replicase amplification and nucleic acid synthesis in general.

THE DISCLOSURES OF THE '338 PATENT

The '338 patent describes methods of detecting nucleic acid sequences that use capture of polynucleotide sequences on a solid phase support and non-specific amplification of the captured polynucleotide. I have been informed that the filing date of the first patent application that discloses this combination of steps and from which the '338 patent claims priority is December 21, 1987 (the "filing date").

I understand that I may be asked to testify as to the level of ordinary skill that is applicable to consideration of the '338 patent. I consider the level of ordinary skill in the art of molecular biology at the filing date of the '338 patent application to have been that of an individual with a Ph.D. in the biological sciences and two years of postdoctoral experience. Such experience would have allowed the individual to develop the skills of a molecular biologist using the techniques of DNA and RNA isolation and characterization, cDNA synthesis, cloning, liquid and solid phase hybridization (including knowledge of the conditions influencing hybrid

formation and stability), affinity chromatography, isotopic and non-isotopic labeling methods, DNA sequencing methods, and nucleic acid amplification, such as by using a DNA polymerase.

Example 7 of the '338 Patent describes non-specific amplification using an RNA polymerase known as Qß replicase. Qß replicase is an enzyme comprised of four polypeptide chains, one of which is encoded in the genome of bacteriophage Qß. The other three polypeptides are encoded in the genome of the bacterium *Escherichia coli*, which Qß infects. The enzyme has RNA-directed RNA polymerase activity and is isolated from *E. coli* infected with bacteriophage Qß.

As of the filing date, one of ordinary skill in the art could not have used the disclosures in the specification of the '338 patent relating to Qß replicase, including Example 7, to amplify heterologous RNA (i.e., RNA that is not bacteriophage Qß genomic RNA or an RNA structurally related to it). Heterologous RNA would include rRNA or RNA transcribed from target DNA. The reasons supporting this opinion are set forth in the following paragraphs.

As of 1987, Qß replicase was known to copy *in vivo* and *in vitro* only Qß genomic RNA (Haruna I and Spiegelman S (1965) "Recognition of Size and Sequence by an RNA Replicase," Proc. Natl. Acad. Sci. U.S.A. 54, 1189-1193) and smaller RNAs generated in Qß-infected *E. coli* that are related to Qß RNA (Kacian DL, Mills DR, Kramer FR, and Spiegelman S (1972) "A Replicating RNA Molecule Suitable For a Detailed Analysis of Extracellular Evolution and Replication," Proc. Natl. Acad. Sci. U.S.A. 69, 3038-3042). This extraordinary template specificity enables Qß replicase to distinguish Qß RNA from the vast number of different RNA molecules that are present in *E. coli*. Thus Qß replicase does not copy other nucleic acids and, consequently, the viral RNA is efficiently replicated after infection. The extraordinary template specificity of Qß replicase is a consequence of two separate interactions that occur between the replicase and its naturally occurring template RNAs. First, the replicase binds strongly to a unique recognition sequence (Vollenweider HJ and Koller T (1976) "Physical Mapping of Qß Replicase Binding Sites in Qß RNA," J. Mol. Biol. 101, 367-377; Meyer F, Weber H, and Weissmann C (1981) "Interactions of Qß replicase with Qß RNA," J. Mol. Biol. 153, 631-660;

Nishihara T, Mills DR, and Kramer FR (1983) "Localization of the Qß Replicase Recognition Site in MDV-1 RNA," J. Biochem. 93, 669-674). Then, product strand synthesis is initiated at a cytidine-rich sequence located at the 3' end of the template (Rensing U and August JT (1969) "The 3' Terminus and the Replication of Phage RNA," Nature 224, 853-856; Mills DR, Kramer FR, Dobkin C, Nishihara T, and Cole PE (1980) "Modification of Cytidines in a Qß Replicase Template: Analysis of Conformation and Localization of Lethal Nucleotide Substitutions," Biochemistry 19, 228-236). Each of these sequences must be present in both complementary RNA strands for exponential amplification to occur.

Many investigators wished to use Qß replicase to catalyze *in vitro* the exponential synthesis of heterologous RNAs. By 1987, a number of schemes had been devised in efforts to circumvent the extraordinary specificity of Qß replicase. Solutions containing manganese were used to decrease the stringency of the interactions between Qß replicase and its template (Palmenberg A and Kaesberg P (1974) "Synthesis of Complementary Strands of Heterologous RNAs with Qß Replicase," Proc. Natl. Acad. Sci. U.S.A. 71, 1371-1375; Obinata M, Nasser DS, and McCarthy BJ (1975) "Synthesis of Probes for RNA Using Qß Replicase," Biochem. Biophys. Res. Commun. 64, 640-647). Primers were used to bypass the normal initiation step (Feix G and Hake H (1975) "Primer-directed Initiation of RNA Synthesis Catalyzed by Qß Replicase," Biochem. Biophys. Res. Commun. 65, 503-509; Feix G (1976) "Primer-dependent Copying of Rabbit Globin mRNA with Qß Replicase," Nature 259, 593-594); Vounakis JN, Carmichael GG, and Efstratiadis A (1976) "Synthesis of RNA Complementary to Rabbit Globin mRNA by Qß Replicase," Biochem. Biophys. Res. Commun. 70,774-782). And polycytidine sequences were added to templates to mimic the required 3'-terminal initiation sequence (Feix G and Sano H (1975) "Initiation Specificity of Poly(cytidylic acid)-dependent Qß Replicase Activity," Eur. J. Biochem. 58, 59-64; Küppers B and Sumper M (1975) "Minimal Requirements for Template Recognition by Bacteriophage Qß Replicase: Approach to General RNA-dependent RNA Synthesis," Proc. Natl. Acad. Sci. U.S.A. 72, 2460-2643).

These strategies were tried with a wide range of heterologous templates, including rRNAs, viral RNAs, and eukaryotic mRNAs. In all cases, the amount of RNA synthesized never exceeded the original amount of template RNA and the products only consisted of complementary strands that remained hybridized to the template strand. By 1983, my laboratory had shown that heterologous oligonucleotides could be inserted at an appropriate site within a naturally occurring Qß template RNA, and the resulting "recombinant RNAs" could be amplified exponentially by incubation with Qß replicase (Miele EA, Mills DR, and Kramer FR (1983) "Autocatalytic Replication of a Recombinant RNA," J. Mol. Biol. 171, 281-295). Ultimately, we showed that recombinant mRNAs could be amplified exponentially in this manner (Wu Y, Zhang DY, and Kramer FR (1992) "Amplifiable Messenger RNA," Proc. Natl. Acad. Sci. U.S.A. 89, 11769-11773).

In 1987, however, no one had any idea how to generate a recombinant RNA from a heterologous RNA. Thus, in 1987, no methods were known in which Qß replicase could be used to exponentially amplify heterologous RNAs. The superficial disclosures of Example 7 of the '338 patent did not enable one of ordinary skill in the art to use Qß replicase to exponentially amplify heterologous RNAs.

In 1980, Thomas Blumenthal published an article that stated that "Qß replicase can transcribe heterologous natural RNA species in the absence of [manganese ions] if sufficient GTP is present." (Blumenthal, T. (1980) "Qß Replicase Template Specificity: Different Templates Require Different GTP Concentrations for Initiation," Proc. Natl. Acad. Sci. U.S.A. 77, 2601-2605, see abstract.) This article was cited in Example 7 of the '338 patent as providing the conditions for exponentially replicating both mRNA and rRNA non-specifically using Qß replicase. In Blumenthal's reported experiments, three synthetic RNAs and two naturally-occurring heterologous RNAs (bacteriophage f2 RNA and rRNA) were transcribed under conditions that varied the concentration of GTP, a nucleotide that is always used by Qß replicase in transcription initiation (see page 2602, column 1). His results showed that the GTP requirement for synthesis was different for each of the five templates tested and was further

changed by the amount of manganese and/or salt ("ionic strength") in the reaction (see page 2602, column 1 to page 2603, column 1). Using radioactive labels, Blumenthal also measured the amount of transcription initiation and the product lengths for the three synthetic templates, which varied depending on the template used (see page 2603, column 1).

In all of these experiments, Blumenthal measured *transcription* of the heterologous RNAs and never showed or even suggested that the reactions resulted in exponential replication of the templates. Blumenthal never showed or implied that the transcripts produced in these reactions would become a template for the enzyme for further replication as Example 7 of the '338 patent states. Even if the conditions described in this article could be used to initiate transcription of heterologous RNAs, it is clear from the article that one skilled in the art would expect highly variable results for each template tested, and would have to engage in extensive experimentation to determine the appropriate conditions for transcription of each heterologous RNA. Even if optimized conditions were determined for *transcription* of each heterologous RNA, the conditions described in this article would not result in exponential replication of the template RNA. Therefore, Example 7 of the '338 patent does not teach one skilled in the art how to achieve exponential replication of heterologous RNA by using Qß replicase, even when combined with the information described in the cited article.

I have been informed that the inventors of the '338 patent cannot state that they attempted to actually practice the method described in Example 7. Many scientists during the period 1974 to 1992 tried to devise ways to exponentially amplify heterologous RNA using Qß replicase. Had the inventors successfully used Qß replicase to exponentially amplify heterologous RNA, that success would have been significant and I believe the inventors would remember it. Had the inventors successfully used Qß replicase to exponentially amplify heterologous RNA, they would have been able to provide adequate information in Example 7 to permit others to practice their method.

CONCLUSION

My overall conclusion is that the disclosure in Example 7 of the '338 patent would not enable one skilled in the art to achieve exponential target amplification using Qß replicase as of the filing date.

In addition to the opinions expressed in this report, I intend to consider and respond to opinions, related to the subject matter of this report, which may be expressed by any expert retained by Vysis, Inc.

August 31, 2001

Fred Russell Kramer

Fred Russell Kramer, Ph.D.